REMARKS

Favorable reconsideration is respectfully requested in view of the preceding amendments and the following comments.

Claims 4 to 17 have been cancelled, as required.

The rejection of claim 1 "under 35 U.S.C. 112 (second paragraph) is respectfully traversed. Applicants submit that the distinguishing characteristics of the claimed strain distinguish it from all other known strains of *Saccharomyces cerevisae.*

The rejection of claims 1 to 3 is also respectfully traversed. Please note that the claims are directed to a strain; Applicants are not claiming a new species.

A deposit has been made under the Budapest Treaty. In support of the foregoing please find herewith a copy of Applicants' submission of October 3, 2002, and confirmation of receipt thereof on October 25, 2002, by the accompanying copy of a letter dated October 28, 2002.

The further rejection of claim 1 "under 35 U.S.C. 112, first paragraph" is also respectfully traversed. Issue is respectfully taken with the unsupported allegation that such claim is a "broad generic claim". It is a very specific claim drawn to a very well defined strain by characteristics which distinguish it from all other known strains. The specified characteristics are adequate to apprise any artisan whether a particular strain falls either within or without the scope of that claim.

The rejection of claim 1 "under 35 U.S.C. 102(b) as anticipated by USP 4,560,659...or, in the alternative, "under 35 U.S.C. 103(a) as obvious over Asturias" is respectfully traversed.

Applicants' (D &M's) strain BPSC-15 invention, being of the same species as the several strains identified by Asturias - *Saccharomyces cerevisae* - [with Asturias mentioning strains *Saccharomyces* L-180, ,L181, L-200, L-208, L-140, and *Saccaromyces cerevisae*. L-169 (hybrid 5-nonflocculent) (column 3, lines 15-20) identified as illustrative useful strains, and strains L-180 and L-181 identified as being "particularly preferred" - also maintained as S.c. CBS-2959 and CBS-1242, respectively, by the CBS culture collection] necessarily has many of the same properties of all *Saccharomyces cerevisae* which primarily consist of strong fermentative ethanolic fermentation of glucose, sucrose, and fructose sugars, but is more exhaustively identified by the taxonomic properties given by Kurtzman and Fell "The Yeasts: A taxonomic study. Elsevier Press, 1998" Applicants are not claiming that their yeast strain produces a

different product than any other *Saccharomyces* yeast strain based ethanolic process (Asturias, Budweiser, etc), but rather a *Saccharomyces cerevisae* yeast based process which produces ethanol far faster (high speed) and under high osmotic pressures (allowing low effluent ethanol production) and to high ethanol concentrations (allowing lower distillation costs); when their teachings are followed, all three properties are novel and useful in the fermentative ethanol production industry.

All strains of *Saccharomyces cerevisae* currently used for batch industrial or beverage ethanol production are characterized by a growth phase, a stationary phase, and a flocculent phase, where having completed the fermentation, the cells begin to clump together and to fall slowly to the bottom of the fermenter (if the fermentation media is not viscous as with whole corn mash). This property of flocculating after completion of the fermentation is important in the potable ethanol / brewing industry, as clear fermented beverages are desired by brewers of wine and beers. (Also discussed on page 4, lines 10 to 20, of Applicants' specification. During the growth phase, the concentration of suspended free (single and budding mother cells increases from the inoculation density, typically 2 to 10 million cells per milliliter (approximately 0.1 to 0.7 g/L yeast d.b.) which increases to 100 to 150 million cells/ml (7 to 11 g/L yeast d.b) by the end of the growth phase. After completion of the fermentation, during the flocculation/clarification phase, the broth appears clear again once the yeast cell density drops below 20 x 10^6 cells/ml (approx. 1.4 g/L yeast d.b.) The *Saccharomyces cerevisae* yeast strains of Asturias follow this same pattern.

The ability of a yeast strain to stay in a self flocculent mode, with large coherent, stable pellets, even when conditions for growth and/or fermentation are optimal (high sugar & nutrients, low ethanol), is a rare quality. Contrasting the floc yeast strain performance of Applicants' BPSC-15 (NRRL 30630) with *Saccharomyces cerevisae* L-180 of Asturias,

Asturias Example 2. Two Step Fermentation of sugar leached from cane 'pith':

Step 1.(Use strain L-180 to ferment sucrose extracted from cane 'pith')
Innoculate: 200 ml water/75 g cane 'pith' with 40 ml of 24 hour innoculum of L-180.
Free yeast density of innoculum of 8.5 g/L d.b.
Ferment: 40 hours @ 30°C
Finish: 26.7 g/L ethanol Productivity of 0.66 g ethanol/Liter hour

Calculation method: final ethanol concentration (converted from g ethanol per 100 ml solution to g ethanol per liter divided by the hours of fermentation time)

Initial solution osmolality: 0.14 Os/kg (45 g/L sucrose solution)
(calculated based on osmolality of 45 g/L sucrose solution - CRC Handbook of Chem. & Physics, 58th Edition - properties of sucrose solution - Table 82, page D-261, or Applicants' Eq. 10)

Initial Free cell density: 1.4 g/L
(converted from g/100 ml to g/L)
Final solution osmolality: 0.55 Os/kg
(calculated based on osmolality of 26.7 g/L ethanol solution - Table 16, page D-227 CRC Handbook or Eq. 10 from D&M)
Final Broth Free cells density: 2.9 g/L d.b.
(converted from g/100 ml to g/L)

Step 2. (Add broth to new fresh 'pith')
Separate pith solids from liquid broth containing ethanol and free yeast @ 2.9 g/L d.b.
Innoculate: Add 169 ml of this broth to 15 g cane 'pith'
Ferment: 24 hours
Finish: 50.5 g/L ethanol Productivity of 0.99 g ethanol/Liter hour
Calculation method: final ethanol concentration [converted from g ethanol per 100 ml solution, and productivity determined as final g ethanol (minus initial ethanol) per liter of fermentation broth divided by the hours of fermentation time]

Initial solution osmolality: 0.69 Os/kg
*[Sum of sugar and ethanol osmolalities (Eq. 3/Eq 10 from D & M) - with initial sugar concentration calculated from conversion efficiency given by Asturias as 0.63 g ethanol per g of sucrose consumed - which would calculate to an initial sucrose concentration of 38 g/L (note: maximum theoretical conversion yield - **Y p/s**- is 0.51g ethanol/g sucrose (100% efficiency), with actual efficiencies ranging from 86 to 90% (Y p/s of 0.44 to 0.46) and a maximal obtainable efficiency of 95% (**Y p/s** of 0.485) suggested by Hodge & Hildebrand (1954) (Industrial Fermentations Ed. by Underkofler & Hickey, 1954]. Ethanol yield (**Y p/s**) depends upon yeast strain, amount of sugar used for cell maintenance, cell growth, and non-ethanol by-products, such as CO_2, glycerol, succinic acid, fusel oils, etc.)*
Initial Free cell density: 2.9 g/L
(From previous fermentation)
Final solution osmolality: 1.12 Os/kg
(calculated based on osmolality of 50.5 g/L ethanol solution - Table 16 page D-227, CRC Handbook or Eq. 10)
Final Broth Free cell density: 5.9 g/L d.b.
(converted from g/10 ml to g/L)

Versus: D & M **Example 6.** Consecutive Batch Fermentation of Molasses with Vinasse Recycle

Multiple Repeated Consecutive Batch Fermentations - using the example shown in Figure

5 w/ 30% vinasses recycle

Feed broth: 350 g molasses, 300 ml vinasses bring to 1 Liter with water
Innoculate: 1 liter feed to 300 ml settled cells
Ferment: 8 hours (as shown in Figure 5)
Finish: 100 g/L ethanol Productivity of 12.5 g ethanol/Liter hour
(Calculation method: final ethanol concentration divided by the hours of fermentation time)
Initial solution osmolality: 2.43 Os/kg
(calculated using Eq. 10 and shown in Table 8 - Osmolality of molasses versus model shown in Figure 3)
Initial free cell denity: 0
(there were no free cells in the solution make-up)
Final solution osmolality: 4.09
(calculated using Eq. 10 and shown in Table 8)
Final Broth Free cell density: less than 0.5 g/L after 30 minutes settling of yeast pellets - as per all the fermentations.

Applicants' CBF fermentation, Example 6 - Figure 5, shows an ethanol productivity of over **10 times** higher than the ethanol productivity shown by Asturias' under inhibitory osmolality conditions averaging **4 times** higher than Asturias Example 2, second step fermentation, while producing a **2 times** higher level of inhibitory ethanol. The free cell density of the residual fermentation broth of the D & M fermentation has **less than 1/10 th** of the free cell density reported by Asturias. It is quite clear that this is not an identical organism.

A strain of microbe is not identical to other strains of the same species, even if it produces the same product, if it has improved desirable properties which have important economic advantages to the production of desired microbial product(s). For example Gallo USP 4,472,504, 1984, developed a strain of *T. reesei* with improved levels of cellulase production. Similarly, Takasawa et al, USP 4,634,670, 1987, and Chahal, USP 5,047,332 report on deposited strains of *T. reesei* which have the property of higher levels of expressed cellulase production over the parent strain(s).

While working on this response, Dale, in an on-line patent search for "*Saccharomyces cerevisae*", found a related patent by Mondal, USP 5,693,526 entitled "Strains of yeast of *saccharomyces cerevisiae* and a process for the preparation of such strains of yeast". In this patent, Mondal et al suggest they have developed two flocculent strains of *S. cerevisae*, which they name MTCC 0001 and MTCC 0002. Mondal states the ability of these strains to produce 7

to 12% ethanol on YEPD (Yeast Extract, Peptone, Dextrose) medium and in Example 1 shows MTCC 0001 fermented 17% dextrose (181 g/L glucose) in 48 hours. Mondal et al. do not give the final ethanol concentration or sugar concentrations, but assuming complete sugar utilization and a normal yield of 0.47 g ethanol/g glucose, we can assume a 'best case' final ethanol concentration of 83 g/L.

Thus we can estimate the performance parameters of Mondal Example 1 as:

Initial solution osmolality: 1.17 Os/kg
(from CRC Handbook Table 22 page D-230 or Eq. 10 of D&M)
Initial free cell denity: 0
Final solution osmolality: 2.0 Os/kg
(calculated assuming 83 g/L ethanol using Table 16, page D-227, or Eq. 10 of D&M)
Final Broth Free cell density not given
Productivity: 1.73 g ethanol/ L hr
(calculated as final ethanol concentration divided by hours of fermentation)

Mondal's Examples 2 and 3 show basically identical performance for MC 0002 (Ex. 2) and a mix of MC 0001 and MC 0002 (Ex. 3).

Thus, while Mondal claims his flocculent strain is osmotolerant, no performance data are provided to corroborate this assertion. No long term stability of the flocs is shown or even claimed. The concentration of free cells in the fermentation broth with the floccs is not given. Applicants' CBF fermentation, Example 6 - Figure 5, shows an ethanol productivity of over **7 times** higher than the ethanol productivity shown by Mondal under inhibitory osmolality conditions averaging **2 times** higher than the Mondal examples. It is quite clear that the strain BPSC-15 of D & M is far superior in performance than MC 0001 or 0002 of Mondal et al., with strain BPSC-15 of D&M actually achieving the 6 goals listed by Mondal et al in "Advantages of the Invention", namely:

1) highly flocculent - BPCS-15 is shown to be stable over time in multiple fermentations with very low released free cell density, while MC-0001 and 0002 have no evidence of stability over extended use or to maintain low free cell density.

2) less sugar is utilized for growth, which should give better yield of ethanol per g sugar: BPSC-15 should give this advantage, however, repeated extremely accurate measures of ethanol and sugar concentration are required to determine yield, and to date,

Applicants have not completed a statistically valid yield comparisons between conventional and BPSC-15 floc strain. However, using the data from Example 4, Table 7, ethanol yields (g ethanol per g sugar converted) can be calculated as 0.486 (95%) (3 hour/stg), 0.478 (93.6%) (2 hr/stage) and 0.492 (96.3%) which are very close to the maximal 95% attainable yield suggested by Hodge & Hildebrand (1954). Mondal et al give no data at all on their conversion yields while claiming that their strains might give some improvements.

3) More osmotolerant. The BPSC-15 strain is able to ferment sugars up to a limiting osmality of 5.0 os/kg while Mondal et al only suggest performance up to 2.0 os/kg.

4) Higher initial sugars can be fermented. D & M show fermentation of 230 g/L glucose to 110 g/L ethanol (Example 4, Table 7, 3 stage continuous conversion of corn syrup to ethanol at 2 hour Residence time per stage - 6 hour total residence time for average productivity of 18.3 g ethanol /L hr) versus Mondal showing fermentation of 183 g/L (17%) glucose and not showing how completely finished the fermentation was at 48 hours or final ethanol concentration but with only a (maximal) productivity of 1.73 g/L hr.

5) Higher ethanol (reduces distillation energy): D & M show a maximal possible ethanol concentration of 150 g/L (Example 7, line 14) versus Mondal et al giving no indication of the ability of their strain besides several unsubstantiated claims of "7 to 12 % ethanol" (Examples 1, 2 & 3) or 58 to 95 g/L ethanol if we assume they are using volume percent ethanol

6) ...reduction in effluent volume: D & M show specifically that by practicing the invention as prescribed, molasses vinasses stillage effluent can be reduced from 13 L per liter ethanol to as low as 3.4 L/ L ethanol (Example 6, lines 7 to 11, with 40% stillage recycle) with molasses feedstock and to 1.7 L/L ethanol with starch/fruit sugars at 70% stillage recycle (Example 7, Table 9) while Mondal et al merely make unsubstantiated suggestions that their strain might give some improvements in reducing effluent volumes.

References

Hodge & Hildebrand "Alcoholic Fermentation of Molassesses Chaper 3. from Industrial Fermentations Ed. by Underkofler & Hickey, Chem Pub. Co.1954

H.R. Stark. Chapter 2. "Alcoholic Fermentation of Grain. Industrial Fermentations, edited by Underkofler & Hickey, Chem Pub. Co. 1954

C. Papazian. "The Life Cycle of Beer Yeast" pages 114-120 "The New Complete Joy of Home Brewing" Avon Books, 1991

A.Nanba et al. Kinetic Analysis for Batch Ethanol Fermenation of Saccharomyces cerevisae. J. Ferment. Technol. Vol 65, No. 3, 277-283. 1987

V. Yarovenko and B. Nakhmanovich. Kinetics of Product Synthesis in Continuous Alcoholic Fermentation. Pure & Applied Chem. Vol 36 No. 3, 1973

The preceding clearly and unequivocally completely distinguishes Applicants' claimed subject matter from any known strain of the involved species. Once advised that such a strain can be produced, it is likely that those skilled in the art would be capable of producing similar strains. The only way Applicants can get any true protection for their invention with the noted distinguishing characteristics is to afford them some scope of protection beyond that limited to the single deposited strain.

Those skilled in the art know how to enhance specific characteristics of a strain by the process clearly set forth on pages 9 and 10 of Applicants' specification. Having provided that information, limiting Applicants' protection to the single deposited strain would merely be an invitation to others to experiment in order to produce a strain which might be minutely distinguished from the deposited strain but certainly would not be the strain to which claim 2 is limited.

Having overcome all outstanding grounds of rejection, favorable reconsideration and allowance of Applicants' claims are in order and are respectfully solicited.

Respectfully submitted,

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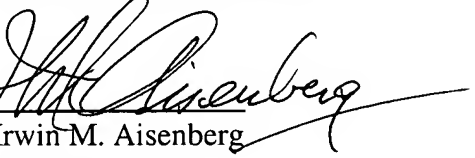
By: 

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